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Biological activity of *Medicago sativa* L. (alfalfa) residues on germination efficiency, growth and nutrient uptake of *Lycopersicon esculentum* L. (tomato) seedlings

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Abstract

Aqueous extract (MSAE) and crude powder (MSCP) of *Medicago sativa* demonstrated inhibitory effects on seed germination, growth and the nutrient uptake of *Lycopersicon esculentum*. The results showed that the extracts brought about considerable inhibition in the germination of tomato seeds and in plumule and radicle length. The allelopathic effect of *Medicago sativa* decreased the germination percentage (GP) of tomato seeds aggravating phytotoxicity (PT) or inhibition percentage with the increase in extract concentration. The extracts also reduced the phytomass of leaves, stems and roots of tomato seedling. The uptake of N, P and K was highly affected and varied drastically by MSCP application. This study revealed that the inhibitory effect of MSAE and MSCP might be due to the presence of some allelochemicals which need further investigation.

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Keywords: Dry matter, germination, *Lycopersicon esculentum*, *Medicago sativa,* nutrient content, phytotoxicity.

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Introduction

Allelopathy which is regarded as a bio-chemical warfare is a natural process involving positive or negative influence of one plant on another through the release of allelochemicals (allelopathins) which are specific biomolecules released mainly through root exudation and biomass decomposition [1, 2] and are recognized to be phytotoxic [3, 4].

Applied allelopathy is believed to have the potential of being of great help for the management of agroecosystems [5]. It has been applied in several agricultural practices, such as cropping systems, weed control, intercropping, nutrient recycling, and low-external input farming practices [6]. Chemicals from the growth of adjacent plants, and therefore the role of allelochemicals in agro-ecosystem have attracted the attention of numerous scientists. Recently, researches demonstrated critical cases of seed and growth inhibition, nutrient uptake and biomass reduction by allelochemicals that influenced crop productivity [7, 8, 9, 10]. Those phytochemicals alter the growth or physiological functions that encounter them during growth. Previous assertion has been suggested that allelochemicals inhibit plant growth by blocking of nutrient reserve, cell division, cell differentiation, ion and water uptake, water stress, phytohormon metabolism, respiration, photosynthesis, enzyme function, signal transduction, as well as gene expression [11,12] thereby caused significant reduction in the growth of plumule and radicle of many crops [13,14].

Alfalfa has been shown to have suppressive effects on both its own species (autotoxicity) and different species (heterotoxicity) which is commonly used in forage crop systems. Saponins (glycosides) are being responsible for alfalfa heterotoxicity and have potential as herbicides [15]. Experimentally, alfalfa root exudates severely inhibited the dry weight of barley (*Hordeum vulgare*) and radish (*Raphanus sativus*) seedlings [16]. Additionally, the aqueous extracts of alfalfa herbage inhibited seed germination and seedling growth of dicotyledonous species more than monocotyledonous ones [17]. Furthermore, the addition of aqueous extract of alfalfa strongly affects the germination efficiency and growth characters of *lepidium sativum* [18].

The main objective of the current study was to investigate the possible allelopathic effects of alfalfa (*Medicago sativa* L., Family: Fabaceae) crop residues on germination, growth and nutrient uptake of tomato (*Lycopersicon esculentum* Mill., Family: Solanaceae).

Materials & Methods

Field observations were set up during summer 2009 and extended to the next season during 2010 in an area of 6.3 hectare cultivated with tomato in traditional cropping systems after harvesting of alfalfa at El-Hammam region; about 75 km southwest of Alexandria city. These observations have confirmed that the performance of this crop species followed alfalfa residues was severely affected.

1.Preparation of *Medicago sativa* aqueous extract

Fresh complete samples (Aerial shoots + roots) of alfalfa were collected from natural agro-fields in summer 2009. The samples were air- dried then cut into 0.5 – 1 cm pieces. Stock aqueous extract was obtained by soaking air-dried plant material in 10% (w/v) distilled water at room temperature (20 ±2^{o}C) for 24 hours with occasional shaking. The mixture was filtered through Whatman No. 1 filter paper and the purified extract was adjusted to pH 6.8 with 1M HCl. Subsequent dilutions (1, 2, 4 and 8%) were prepared from the stock solution in addition to the control (distilled water).

2. Germination bioassay

Twenty five seeds of tomato were arranged in 9-cm diameter Petri-dishes lined with two discs of Whatman No.1 filter paper under normal laboratory conditions with day temperature ranging from 19-22^{o}C and night temperature from 12-14^{o}C. 10 cm of each level of the alfalfa extract (1, 2, 4 and 8%) were added daily to three replicates. Before sowing, the seeds were surface sterilized with 2% sodium hypochlorite for 2 minutes then rinsed four times with distilled water. The sterilized seeds were soaked in aerated distilled water for 24 hours. Germination percentage (GP), plumule (PL) and radicle (RL) length were recorded after 15 days at the end of the experiment. Seed germination index (SGI) was calculated according to the following equation [19].

$$SGI = \sum Ti\ Ni / S$$

Where,
Ti = is the number of days after sowing
Ni = is the number of seeds germinated on day i
S = is the total number of seeds planted

Likewise, phytotoxicity (PT) of the target species extract was expressed as a percentage of growth (germination) of the test species in different concentration levels with respect to water control. Higher values indicate lower toxicity [20].

$$\text{Phytotoxicity (PT)} = [1- (\text{allelopathic/control}) 100]$$

3. Pot experiment

Pot experiment was carried out to test the effect of different concentrations of *Medicago sativa* crude powder (MSCP) (w/w) on dry matter accumulation, nutrient concentration and uptake in sandy loam soil. Seeds of tomato were obtained from the Breeding Program of the Agricultural Research Center, Giza, Egypt.Ten seeds from the investigated species were sown in plastic pots (diameter 20cm x height 22cm) with sandy loam soil completely mixed with electrically crushed crude powder of alfalfa plant (w/w) make available the alfalfa crude powder (MSCP) concentrations of 1, 2, 4 and 8%. The experiment was performed under normal laboratory conditions with day temperature ranging from 19 -22^{o}C, light duration was 12 hours and light intensity was 1350 foot-candle

(F.C.). The plants were watered every two days on the average with normal tap water. Pots were carefully irrigated from the top the first time and sub-irrigated thereafter to avoid seedling disturbance. The amount of water corresponding to average soil-plant-transpiration calculated from weight loss over 24-hours for three replicates. Seedlings of tomato were harvested one month after planting. One treatment was run as control without any percent of the crude powder. A definite number of complete morphologically homogenous tomato seedlings from each treatment were harvested and washed by running water to remove adhering soil particles, followed by distilled water. The samples were separated into different organs which were dried at 65^{o}C till constant weight to determine the weights of stem (SW), leaf (LW), root (RW) and total weight (TW). A part of the dried samples were ground in a Wiley Mill to pass 1.0 mm^2 screen. Nitrogen (N), phosphorus (P) and potassium (K) were determined according to procedures described by Allen *et al.* [21]. At the end of the experiment (one month after sowing) the total nutrient uptake (mg plant^{-1} month^{-1}) at each MSCP concentration was calculated according to the following equation:

Total nutrient uptake = NC * TDW.

Where

NC: Nutrient concentration.

TDW: Total dry weight.

4. Statistical treatments

Data concerning the effect of different concentrations of *Medicago sativa* aqueous extract (MSAE) and *Medicago sativa* crude powder (MSCP) on germination and some growth parameters beside nutrient concentration and uptake of tomato plants were subjected to standard analysis of variance (ANOVA) using the COSTAT 2.00 statistical analysis software manufactured by CoHort Software Company [22]. Significance of differences was accepted at P≤0.05.

Results

1. Germination efficiency and growth

Data of germination percentage (GP), seed germination index (SGI) and phytotoxicity (PT) of tomato plant are illustrated in (Fig. 1). GP of seeds was significantly (P≤ 0.05) affected upon applying different concentrations of *Medicago sativa* aqueous extract (MSAE). Commonly, the percentage decreased with the increase in MSAE concentrations. The value achieved at control and 1% MSAE concentration was about 82% compared to 38.3% at 8% (maximum MSAE concentration). Regarding SGI, the value decreased distinctly as MSAE concentration increased. This reduction was statistically (P≤ 0.01) highly significant. Initially, SGI began with value of about 12.3 at both control and 1% MSAE concentration. Continuously, lower SGI values (7.9 and 5.7) were obtained at 4 and 8% MSAE concentrations, respectively. PT was nil at 1% MSAE concentration. Correspondingly, it attained a value of about 52.9 at 8% MSAE concentration, indicating a gradual enlargement in the PT as more allelopathic extract concentration is increased.

Data concerned with PL and RL are illustrated in (Fig.2). All allelopathic concentrations have significantly (P≤ 0.01) affected both measurements. The immense negative response of the plumule growth (7.3 cm) was marked at 8% concentration compared to 18.3 cm recorded at control level. Interestingly, 1% MSAE concentration was regarded as a stimulating concentration for tomato seedling growth (20.6 cm) compared to the control. A gradual decrease from the control (9.8 cm) in RL was observed along gradual MSAE concentrations (3.8 cm at 8% MSAE).

2. Dry Matter accumulation

Data exhibited an allelopathic action of *Medicago sativa* crude powder (MSCP) on some dry weight estimates [stem (SW), leaf (LW), root (RW) and total (TW)] of tomato seedlings as well as their statistical elucidation (Table 1). Dry weight estimates are highly correlated (P≤ 0.01) with MSCP concentration which pronounced a suppressive influence. Maximum seedling TW (5.6g) achieved at control level has demonstrated an obvious reduction to 1.8 at 8% concentration. Fractionally, SW, LW and RW attained values of about 2, 2 and 1.6g at control level which subsequently reduced to about 0.6, 0.7 and 0.5g at the maximum concentration level (8% MSCP concentration).

3. Concentration and uptake of plant nutrients

The concentration (mg g^{-1} d.wt.) of nitrogen (N) phosphorous (P) and potassium (K) in tomato seedlings was highly affected by the application of different concentrations of MSCP (Table 2). The three elements have pointed out a gradual reduction at variant MSCP concentrations. The concentrations of three elements were about 10.5, 0.71 and 18 mg g^{-1} dwt at control level while at 8 % concentration level they were reduced to about 2, 0.26 and 5 mg g^{-1}d.wt., respectively. The uptake of NPK was highly affected by MSCP application (Fig.3). Highly N uptake has varied drastically with the applied MSCP concentration. At control, it was about 58.8 mg plant^{-1} month^{-1} while the least uptake was recorded at 8% concentration (3.6 mg plant^{-1} month^{-1}). Surprisingly, 1% MSCP concentration has enhanced N uptake (87.1 mg plant^{-1} month^{-1}) relative to the control. Frequently, at 8% MSCP concentration, reduction in N uptake process was more evident (20.4 mg plant^{-1} month^{-1}). The value of P uptake at control level was about 3.97 mg plant^{-1}month^{-1} At 8% concentration, the values were reduced to about 0.47 mg plant^{-1} month^{-1}. The control values of K uptake were 100.8 mg plant^{-1} month^{-1}. Perceptibly; MSCP concentrations have showed an inhibitory effect on K uptake process. Continually, the values 67.2, 49.4, 20 and 9 mg plant^{-1} month^{-1} were obtained at 1, 2, 4 and 8% MSCP concentrations.

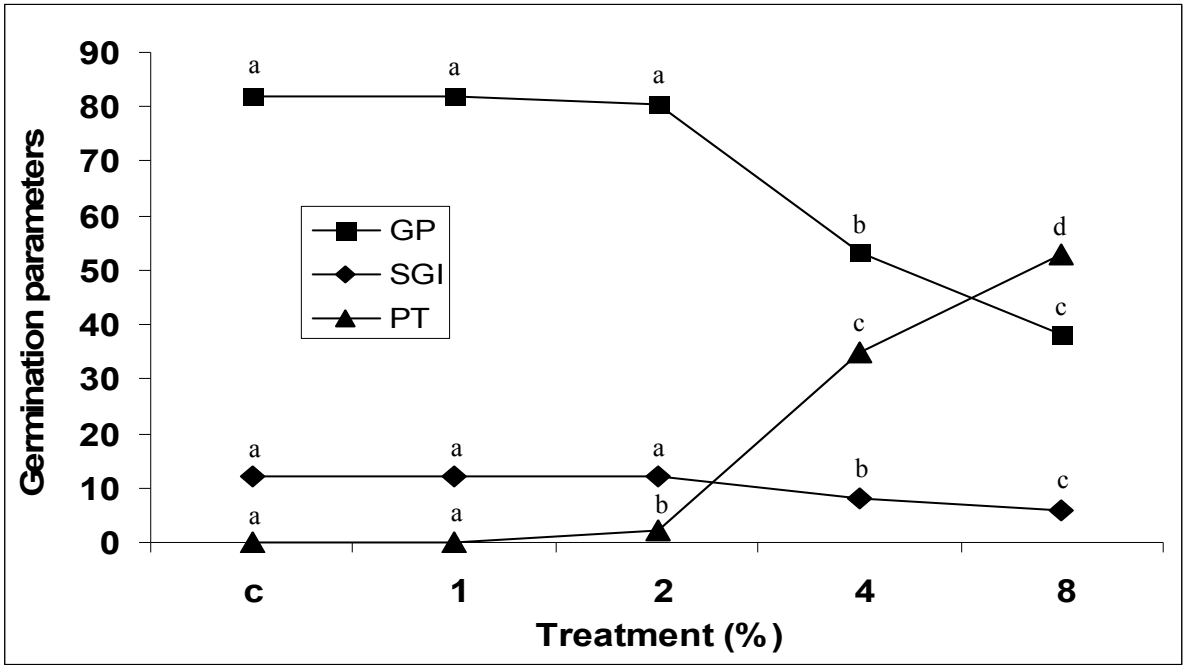

Fig. 1. Allelopathic effect of different concentrations of *Medicago sativa* aqueous extract (MSAE) on germination percentage (GP), seed germination index (SGI) and phytotoxicity (PT) (15 days after sowing) of *Lycopersicon esculentum* seedlings. (Data are means of three replicates). Different letters for each parameter indicate a significant difference at the 0.05 level of probability as evaluated by ANOVA test.

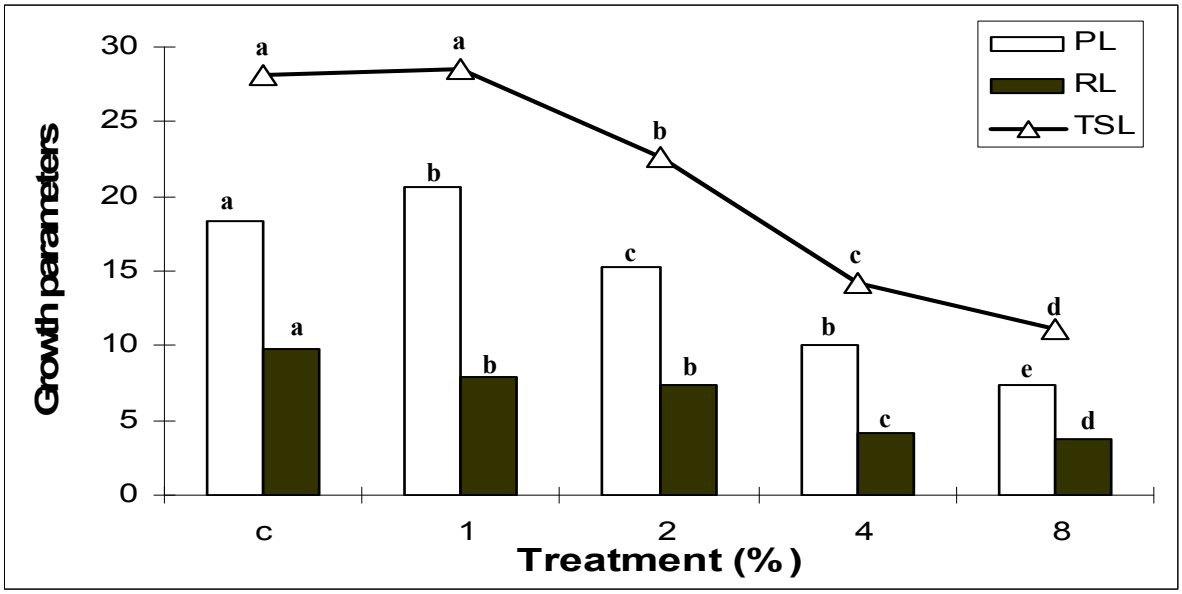

Fig. 2. Allelopathic effect of different concentrations of *Medicago sativa* aqueous extract (MSAE) on plumule (PL) and radicle (RL) length (cm) as well as total seedling length (TSL) (15 days after sowing) of *Lycopersicon esculentum* seedlings. (Data are means of three replicates). Different letters for each parameter indicate a significant difference at the 0.05 level of probability as evaluated by ANOVA test.

Table 1: Allelopathic effect of different concentrations of Medicago sativa crude powder (MSCP) on dry weight (g) (30 days after sowing) of Lycopersicon esculentum seedlings. (Data are means of three replicates). Different letters for each parameter indicate a significant difference at the 0.05 level of probability as evaluated by ANOVA test.

Treatment (%)	*Stem dry weight (SW)*	*Leaf dry weight LW) (*	*Root dry weight (RW)*	**Total dry weight (TW)**
C	2.0^{c}	2.0^{b}	1.6^{b}	5.6^{c}
1	1.4^{b}	2.0^{b}	1.4^{b}	4.8^{bc}
2	1.4^{b}	1.7^{b}	1.2^{ab}	4.3^{b}
4	0.8^{a}	1.1^{ab}	0.6^{a}	2.5^{a}
8	0.6^{a}	0.7**a**	0.5**a**	1.8^{a}
LSD	0.363	0.670	0.670	0.890

Discussion

The current study suggested that the germination percentage (GP) of tomato (*Lycopersicon esculentum* Mill.) seeds was reduced (about 47% of the control) when 8% concentration of *Medicago sativa* aqueous extract (MSAE) was applied. These results are in agreement with those previously reported by Nakahisa *et al*. [23], who found that aqueous extracts of alfalfa shoots reduced germination of alfalfa and radish seeds (35% and 80% relative to control, respectively) only when a high concentration was used. The reduction was concentration dependent. These assessments are also in a harmony with Chung and Miller [24] who reported that aqueous extracts of alfalfa and hairy vetch reduced corn and soybean seed germination**.**

Results of seed germination index (SGI) indicated that a gradual reduction of SGI as a response to the regular applying of higher MSAE concentration levels was attained, supporting the previously documented GP implications. Phytotoxicity (PT) or inhibition percentage increased gradually with the increase of MSAE concentration; the maximum PT (about 53%) was recorded at 8% MSAE concentration. Alagesaboopathi [2] reported that leaf, stem and root extracts of *Andrographis paniculata* inhibited the seed germination of *Seasmum indicum*. Highest inhibition (78%) was observed with concentrated leaf extract.

Growth bioassays are often more sensitive than germination bioassays [25, 26, 2, 10]. Fuentes *et al*. [27] observed that seed germination has been regarded as a less sensitive method than plumule and radicle length when used as a bioassay for the evaluation of phytotoxicity**.** Bioassays of germination, radicle, plumule and coleoptile growth are used to test the allelopathic potential of crop species [28]. Therefore, elongation of the hypocotyl or coleoptiles can be used in concurrence with GP.

It was inferred from the present study that radicle growth of tomato plant was found more sensitive and responds more strongly to the increase in MSAE concentration than the plumule did. The reduction may be due to phytotoxic activity of phytochemicals present in aqueous extracts of alfalfa. Evidently, a limited stimulatory effect at 1% MSAE concentration was achieved. This finding is in accordance with a study stated by El-Darier and Youssef [29]; who stated that there was an increase in plumule growth rate of *Lepidium sativum* till 50% alfalfa extract after five days of experiment.

The detrimental effects of allelochemicals on plant growth have been reported by several authors [30, 31, 32, 33]. Leachates from plants have been shown to suppress early seedling growth [34]. Earlier, inhibition of wheat (*Triticum aestivum*) radicle growth was positively associated (r=0.66) with concentrations of total phenolics contained in sorghum (*Sorghum bicolor*) plant parts [35]. Similar results were obtained on broad bean and barley [9, 10]. The MSAE also caused significant reduction in tomato seedling growth. The extracts not only reduced the plumule and radicle length of seedlings but also reduced the leaf, stem and root dry weight. The reduction of biomass was correlated with reduction in seedling growth. The reduction may be due to stunted and meager vegetative growth of seedling. Similar findings were also obtained by several studies [36, 37, 38, 39, and 40]. Earlier**,** Terzi *et al*. [41] emphasized that the dry weights of root and stem of cucumber seedlings was influenced negatively by decomposed walnut leaves and juglone, depending on the concentration.

In an earlier work, the allelopathic effect of *Chenopodium album* and *Senecio vulgaris* on tomato was evaluated. Leachates of the first species significantly reduced shoot fresh and dry weights and the accumulation of N, P, K, Ca and Mg of tomato shoots [42]. Phenolics are capable of interfere with the uptake of nutrients, and can also affect the rates of nutrient cycling [43]. Data of tomato plant only demonstrated that K concentration exhibited a gradual

decrease correlated with escalating MSCP concentrations.

The interference with nutrient uptake and subsequent reduction in nutrient accumulation is one of the most effective mechanisms of phenolic compounds action [44]. Moreover, allelochemicals can alter the rate at which ions are absorbed by plants. A reduction in both macro- and micronutrients are encountered in the presence of phenolic acids [45]**.** El-Darier [7] confirmed this concept where allelopathic compounds released from *Eucalyptus* leaves significantly suppressed the uptake of N, P and K in broad bean and corn plants.

Conclusion

It can be concluded that alfalfa water extract at higher concentrations suppressed the germination, root and shoot growth and nutrient uptake of tomato seedlings and this suppression was possibly due to the presence of allelochemicals in the donor plant. Although it was a preliminary laboratory study yet it provided encouraging results and basis for future research.

Table 2: Allelopathic effect of different concentrations of *Medicago sativa* crude powder (MSCP) on concentration (mgg^{-1}d.wt.) of nitrogen (N), phosphorus (P) and potassium (K) (30 days after planting) of *Lycopersicon esculentum* seedlings. (Data are means of three replicates). Different letters for each parameter indicate a significant difference at the 0.05 level of probability as evaluated by ANOVA test.

Treatment (%)	*Nutrient Concentration (mgg^{-1}d.wt.)*		
	N	**P**	**K**
C	10.5^{d}	0.71^{c}	18.0^{d}
1	9.5^{cd}	0.75^{c}	14.0^{cd}
2	8.0^{c}	0.62^{bc}	11.5^{bc}
4	5.5^{b}	0.52^{b}	8.0^{ab}
8	2.0^{a}	0.26^{a}	5.0^{a}
LSD	1.84	0.136	2.377

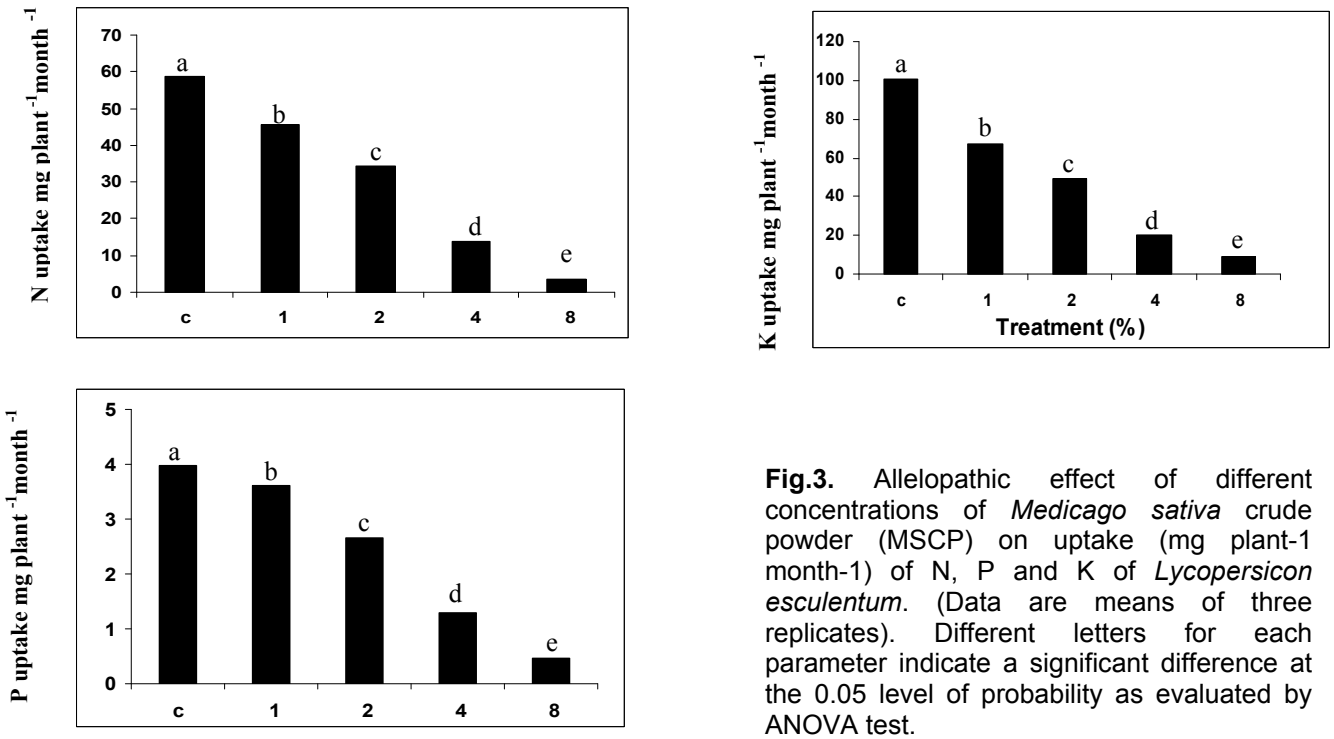

Fig.3. Allelopathic effect of different concentrations of *Medicago sativa* crude powder (MSCP) on uptake (mg plant-1 month-1) of N, P and K of *Lycopersicon esculentum*. (Data are means of three replicates). Different letters for each parameter indicate a significant difference at the 0.05 level of probability as evaluated by ANOVA test.

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